The Action of "Peptone" on Blood and Immunity thereto.

By J. W. Pickering, D.Sc., and J. A. Hewitt, Ph.D., B.Sc.

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(From the Department of Physiology, University of London, King's College.)

The current view on the cause of the non-coagulability of the blood after the injection of "peptone" is that the injected material stimulates the liver (or the liver plus other organs) to form an excess of a substance named antithrombin. The liver is considered further to form antithrombin under normal conditions in amount sufficient to maintain the fluidity of the blood. The statement that "peptone" has little or no anticoagulant action on shed blood (except in high concentrations) is also generally accepted. Immunity to the anticoagulant action of "peptone" which follows its slow injection is commonly ascribed to hepatic activity.

The experiments recorded in this paper show that these views can no longer be held.

On the supposed Formation and Secretion of Antithrombin by the Liver.

Contjean (1), after ligature of the abdominal arterial trunks, and Gley and Pachon (2), after ligature of the lymphatics of the liver, found that injected "peptone" had no anticoagulant action. Starling (3) and Delezenne (4) could not confirm these observations, while Denny and Minot (5) have shown that repeated electrical stimulation of the cœliac plexus or its hepatic branches fails to increase the coagulability of the blood.

The first systematic attempts to prove that the liver secreted an antithrombic substance appear to have been made by Hédon and Delezenne (6), who, after establishing an Eck's fistula in a dog and, as far as possible, removing the liver, failed to reduce the coagulability of the blood by the intravascular injection of "peptone." These investigators concluded that something secreted by the liver was the cause of this result. In each of the two experiments described, some hours elapsed after the establishment of the fistula and before the liver was incompletely excised, and a further hour passed before the injection of "peptone." Delezenne (7) reported four similar experiments (of which only one is given in detail), and here also there is a considerable time-interval, in this case 5 hours, between the end of the operation and the injection of the "peptone." In the 26 minutes immediately following the injection of the "peptone" five samples of blood coagulated rapidly.

The common feature of these experiments is the great length of time during which the animals were under anæsthesia, leading to a decrease of oxygen (Buckmaster and Gardner, 8) and a corresponding increase of carbon dioxide in the blood.

Prior to the work of Hédon and Delezenne (*loc. cit.*), Lahousse (9) had shown that in peptone blood there is an extreme diminution of carbon dioxide, while Fano (10) and Wooldridge (11) had demonstrated that the passage of carbon dioxide through peptone plasma induces coagulation. Wright (12) has also shown that an increase of carbon dioxide in normal circulating blood augments its coagulability.

The following experiments* show that the retarded coagulability of peptone blood in animals intact, except for pithing, or in animals in which the liver is not acting, may be diminished or annulled by excess of carbon dioxide; the general result is that when the vitality of the animal is impaired this inevitably occurs with both moderate and larger doses of peptone.

In these and the subsequent experiments all animals were anæsthetised with A.C.E., pithed, and (except in Nos. 14–17) the anæsthetic was discontinued for at least a quarter of an hour before observations were made on the blood. All blood was withdrawn through evenly paraffined cannulæ. Artificial respiration (except in Nos. 14–17) was employed throughout. The animals were kept warm. The "commencement" of clotting was taken to be when the first change towards coagulation was observed; "completion" of clotting when the vessel containing the blood could be inverted without spilling. All glass vessels were cleaned with hot hydrochloric acid, caustic soda, alcohol and ether. Dust was as far as possible excluded. The percentages of "peptone" are necessarily approximate, since commercial specimens, as used by different investigators, vary enormously in composition. In the present work one preparation was employed throughout.

The results obtained in Experiments 1, 2 and 3 are expressed graphically. The points on the upper graph indicate "completion" of clot, those on the lower graph "commencement" of clot.

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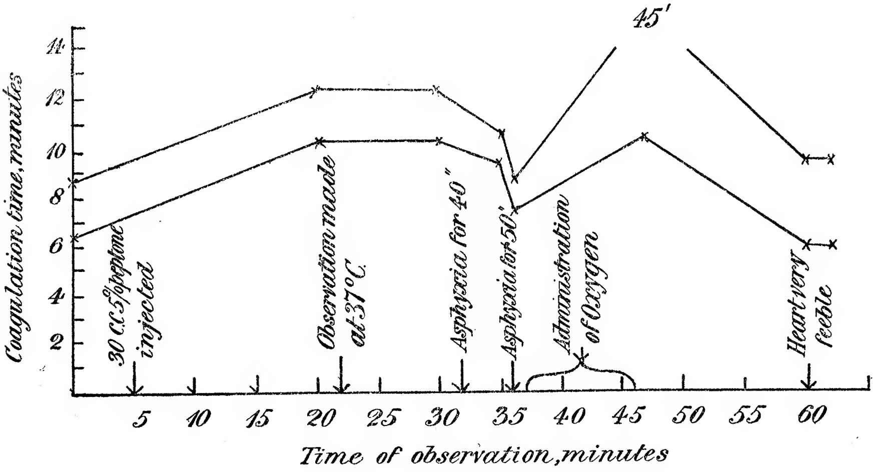
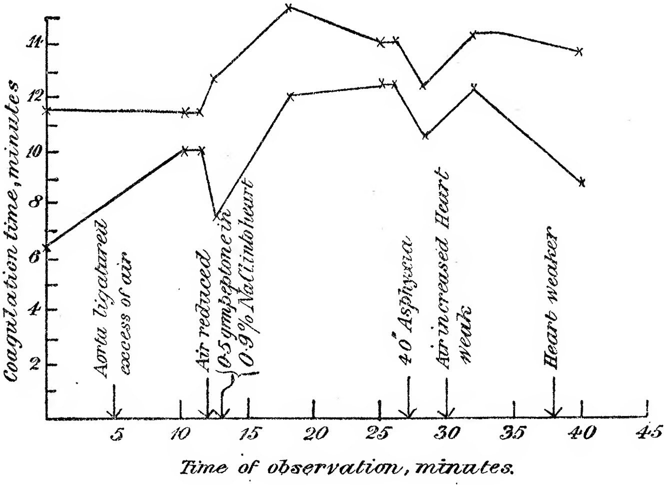
* Cats have been used in the experiments now recorded because their susceptibility to "peptone" lies midway between that of the dog, which is markedly susceptible, and the rabbit which is exceptionally resistant.

Experiment No. 1.—Pithed cat. Peptone injected into right jugular.



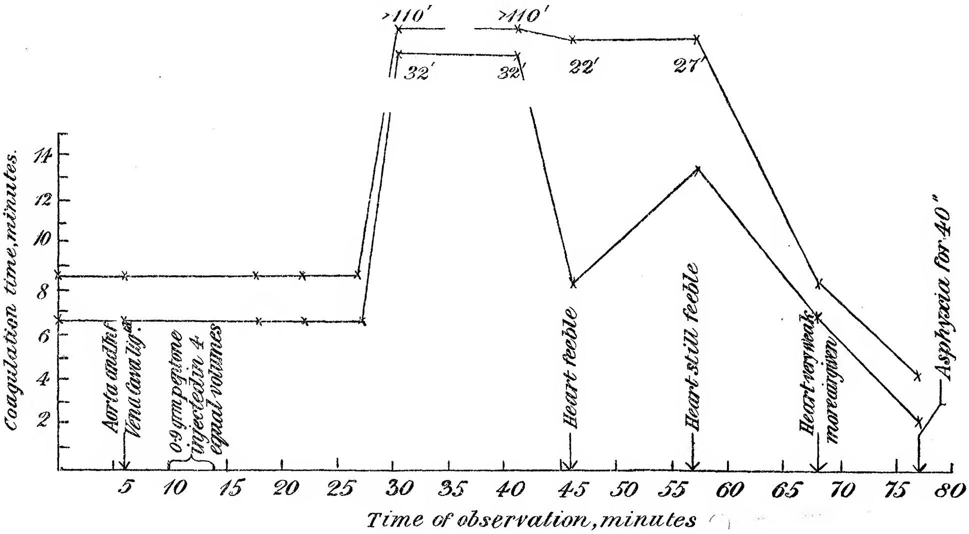
Note.—The clot at 47′ was very loose. From 60′ onwards the heart was weak.

Experiment No. 2.—Pithed cat. Aorta ligatured.



Note.—At 30′ and 41′, the blood was not completely clotted after 1 hour 50 minutes. *Post mortem* examination showed in this and in other experiments the ligatures intact.

Experiment No. 3.—Pithed cat. Aorta and inferior vena cava ligatured.



Experiment No. 4.—Cat, pithed. Artificial respiration. Aorta and inferior vena cava ligatured. 1·625 grm. of "peptone" was injected rapidly into the heart over a period of 4 minutes. Five minutes after the injection of the "peptone," coagulation time showed a retardation of 20 minutes in commencement of clotting, a complete gelatinous clot appearing 2 minutes 40 seconds later. This apparently liquefied, but further clots appeared in the liquid during the subsequent 2 hours, and the fluid was completely clotted on the following morning. While all other results have been repeated, this is the only occasion in these experiments where the phenomenon described in the dog by Doyon has been observed. Other samples of blood from this animal behaved similarly to those in Experiment No. 3, the increase in concentration of "peptone" used not causing any substantially greater inhibition than was evident in No. 3. The passage of carbon dioxide through the peptone blood from this animal gave typical clotting, as did dilution with distilled water.

The total time taken in No. 1 was 1 hour 10 minutes, in No. 2 1 hour 22 minutes, and, moreover, no anæsthetic was being respired. Hédon and Delezenne's experiments occupied "some hours," while in Delezenne's observation no less than 5 hours elapsed from the commencement of the operation till the observation of the coagulation time of the peptone blood. During these protracted periods a volatile anæsthetic was being administered and no attention was paid to the effect of a possible increase of the concentration of carbon dioxide in the circulation, under conditions liable to promote it. So far as the present writers are aware this criticism applies to all experiments in which "peptone" has been injected into animals with damaged or more or less excised livers.

Of the facts unexplained by the hypothesis that the secretion by the liver of antithrombin is the cause of the fluidity of normal circulating blood and of the retarded coagulability of peptone blood, it is noteworthy that the massive injection of thrombin does not produce thrombosis, Wooldridge (13). This was confirmed by Mellanby (14) who injected, without thrombosis, sufficient thrombin into a cat to coagulate 2 litres of blood. After the intravascular injection of thrombin, Davis (15) found the coagulation time of shed blood to be variable, sometimes slightly lengthened at other times shortened. Unfortunately Davis recorded as "coagulation times" only the time of completion of the clot. This may be a variable period when the times of commencement of clotting are approximately constant (Nos. 1–4). Moreover, Davis' variations in coagulation times are not greater than can be accounted for by alterations in the relative concentrations of carbon dioxide and oxygen in the blood shed. Apparently this and the corresponding hydrion concentration did not engage his attention. Davis explains the slightly decreased coagulability following the injection of thrombin by suggesting that thrombin produces an excessive secretion of antithrombin, while Howell (16) maintains that thrombin, or prothrombin is a hormone which stimulates the secretion of antithrombin. Davis frankly admits that if antithrombin exists in the circulation and is a genuine antibody, secreted as a protection against thrombin, then according to all analogies the presence of thrombin should lead to its production.

Nolf (17), however, denies that the intravascular injection of thrombin leads to the formation of antithrombin. Further doubt is cast on the accepted hypothesis by the recent work of Arthus (18), who observed that delay occurs in the coagulation of shed blood after the injection of the venom of *Crotalus adamanteus* into immunised, non-immunised, and even anaphylactic rabbits. This not only happened in whole animals, but in those in which the hepatic blood supply had been cut off.

Two views have been advanced in this connection, viz., that of Martin (19), and of Barratt (20), where the active fraction of snake venom is considered to be a true fibrin-ferment (thrombin), and that of Mellanby (21), that it is a kinase which, when rapidly injected, causes the rapid formation of thrombin. It is evident from the experiments of Arthus, that the "negative phase" of coagulation following either the rapid injection of minimal doses of venom, or the slow injection of larger doses, is not produced by the secretion by the liver of antithrombin, which combines with thrombin, either existing in, or produced by, venom. This conclusion falls into line with the recent observation of Pickering and Hewitt (22), that the slow addition, drop by drop, of tissue extract (nucleo-protein), prepared from kidney, to unsalted bird's blood *in vitro*, produces a " negative phase " in its coagulation, and is explicable by the suggestion that the " negative phase " of blood coagulation is a physical process involving an adsorptive union of the tissue extract, the substances forming the alkali reserve and fibrinogen, and is in its essence closely akin to the following *in vitro* reactions: the Dansyz reaction with the toxins and antitoxins of diphtheria and ricin (Dansyz, 23), the variations in the toxicity incidental to the fractional neutralisation of arsenious acid by ferric hydroxide (Hewlett, 24), the " negative phase," or temporary inhibition of the precipitation of gelatin by the slow addition of either alcohol or ammonium sulphate, and to the similar phenomena following the addition of electrolytes to certain inorganic sols (Spring, 25, Höber and Gordon, 26, Paine, 27, Galecki, 28, Burton, 29).

Arthus concluded that in poisoning by proteotoxins, the liver is either not the organ which produces anti-thrombin, or is not the only organ. Doyon (30) immediately perceived the significance of Arthus' work, and, adopting his methods, injected " peptone " into dogs in which the hepatic circulation had been ligatured. Again, no attention was paid to the probable increase of carbon dioxide in the blood during the experiments in which anæsthetics were employed. Doyon states that, in the cases he observed, the speed of clotting of shed blood was normal, but the clots were soft and completely dissolved on standing, so that the blood again became fluid. This result Doyon ascribed to the action of anti-thrombin formed in parts of the body other than the liver, and reverted to Contjean's views (*loc. cit.*), which have also been supported by Popielski (31).

It is, it is thought, clear that Doyon's clots are not true fibrin clots, as such do not re-dissolve *in vitro*. In a recent paper (Pickering and Hewitt (*loc. cit.*)) evidence has been brought forward that the first stage of coagulation of mammalian and frog's blood, surrounded by oil, is the formation of a reversible gel, which is soluble in excess of tap water. In the case of frog's blood this stage may, when undisturbed, persist for several hours, but if thromboplastic material is added to it a typical clot is formed in a few minutes. It therefore appears possible that " peptone," as administered by Doyon with the liver out of circulation, retarded the coagulation of the blood so that it remained in the first stage of the process. Only in one of the series of experiments now recorded, in which " peptone " was injected into both intact cats and into cats deprived of hepatic circulation, has any evidence been noted of the temporary formation of a gel which dissolves on standing (No. 4). It has consequently been impossible to investigate any relationship to hydrion concentration of this transient but significant phenomenon.

The earlier observations on the effect of the injection of "peptone" into animals with either impaired or partly excised livers, have formed the basis for a vast superstructure of hypotheses. They remain crucial only so long as the anticoagulant action of "peptone" has not been observed in animals when the liver is not acting.

Employing a somewhat simpler operative technique to deprive the liver of circulating blood with a view of avoiding the prolonged interval before the injection of "peptone" (with the consequent accumulation of carbon dioxide), the results given in experiments, Nos. 2, 3 and 4 above, demonstrate decisively that *retardation of coagulation of blood consequent to the rapid intravascular injection of moderate amounts of "peptone" can be produced under conditions which preclude the participation of the liver.*

Stress is laid on the importance of working with pithed animals, and in allowing at least 15 minutes to elapse after pithing and the discontinuance of the anæsthetic before the injection of "peptone," so that any action of the anæsthetic on the coagulability of the blood may be eliminated. It is also advisable to regulate the air supply to the animals so as to maintain, as long as possible, normal oxygenation.

On the action of "peptone" on blood in vitro.

The well known observation of Schmidt-Mulheim (32) that very large quantities of "peptone" are necessary to retard the coagulation of blood *in vitro* is commonly regarded as showing that "peptone" has no specific action on shed blood. Among the earlier observers, Afanassiew (33), however, stated that blood shed direct from the artery of a dog, without exposure to the air, into 1·225 — 1·5 per cent. "peptone," and kept at 40°, remained fluid. Unfortunately the details of the experiment are meagre. Pollitzer (34) stated that 2 per cent. of heteroalbumose inhibited blood clotting *in vitro*, while Halliburton (35) recorded that the same substance also delayed the coagulation of salted plasma. Working with lymph from the thoracic duct which clotted, at room temperature, in from 10 to 15 minutes, Shore (36) found that when relatively large quantities of "peptone" were added *in vitro* the clotting was as rapid as in pure shed lymph, but if smaller amounts of "peptone" were used retardation occurred. In one experiment in which the amount of "peptone" was 0·0377 per cent., the lymph remained fluid for 24 hours.

Camus and Gley (37) reinvestigated the effect of adding "peptone" to shed blood, and stated that from eleven to fifteen times more "peptone" is required to produce retardation of clotting *in vitro* as compared with the amount necessary to give this result *in vivo.* Thus the original views of the non-specificity of the action of "peptone" on shed blood gained general acceptance.

The present authors find that when blood is shed into "peptone" without precautions to preserve it from changes towards coagulation, as in the earlier work, then anticoagulant action is absent, except when the "peptone" is present in relatively large amount. The following experiments show that different results are obtained when blood is shed through an evenly paraffined cannula from an animal respiring air.

No. of experiment	5.	6.	7.	8.	9.	10.
Percentage of "peptone" in blood	p.c. 0·3	p.c. 0·325	p.c. 0·6	p.c. 1·0	p.c. 1·0	p.c. 3·5
	′ ″	′ ″	′ ″	′ ″	′ ″	′ ″
Coagulation times of control blood	7 45	5 55	7 45	7 45	5 55	7 25
	11 45	8 10	11 45	11 45	8 10	8 45
Coagulation times of "peptone" blood	13 20	45 20	16 30	14 45	21 4	?
	16 40	48 50	20 10	23 40	23 40	?
Coagulation times of "water" blood	8 10	6 5	8 10	8 10	8 10	6 5
	10 15	7 55	10 15	10 15	10 15	7 55

Notes.

1. "Water" blood is normal blood to which a volume of water or in No. 6 of 0·9 per cent. NaCl equal to that of the peptone has been added.
2. The upper of the two coagulation times is that of "commencement," the lower that of "completion" of clotting.
3. The blood was arterial and was withdrawn into glass vessels through paraffined cannulæ. The animals were pithed and were breathing air only.
4. The "peptone" in Experiment No. 6 was dissolved in 0·9 per cent. sodium chloride; in all others in this series in distilled water.
5. Room temperature was 13–14° C.
6. The "peptone" blood in No. 10 remained fluid during the next day.

Experiment No. 5 shows that blood withdrawn from the carotid of a cat through a carefully paraffined cannula into a clean glass vessel, and there mixed immediately with "peptone" dissolved in distilled water, exhibited a distinct retardation of clotting. This delay amounted to 5 minutes 25 seconds in the time of commencement, and 4 minutes 55 seconds in the time of completion of clotting; the amount of "peptone" in the blood was only 0·3 per cent. As is seen from experiments 7, 8, 9, and 10 with greater concentrations of "peptone," the inhibition of clotting increased. When solution is effected in 0·9 per cent. sodium chloride as in Experiment No. 6, the inhibition of coagulation is still more marked. Thus with a concentration of 0·325 per cent. of "peptone" in the blood a delay of 39 minutes 25 seconds in the commencement of clotting was observed, while the delay in completion was 40 minutes 40 seconds. The addition of corresponding amounts of 0·9 per cent. sodium chloride to shed blood did not produce any appreciable variation in the coagulation time. A comparison of Experiments Nos. 8 and 9 with No. 6 shows that the retarding effect of a concentration of 1 per cent. of "peptone" dissolved in distilled water was actually less than that of 0·325 per cent. dissolved in normal saline. This result falls into line with the observation of Howell (38) that while dilution of peptone plasma with water induces coagulation, dilution with 0·9 per cent. sodium chloride has no such effect.

In the next three observations, endeavours were made, with success, to prolong the anticoagulant action of small amounts of "peptone" *in vitro* by reducing the disturbance to the colloidal complexes of the plasma incidental to the shedding of blood.

	Control blood on glass.		Control blood on paraffin.		0·3 p.c. peptone blood on paraffin.		0·3 p.c. peptone blood on paraffin.		0·6 p.c. peptone blood on paraffin.	
	′	″	′	″	′	″	′	″	′	″
Time of adding "peptone"...	0	0	0	0	0	0	0	0	0	0
Time of "commencement" of clotting	8	55	24	20	32	40	34	45	45	20
Time of "completion" of clotting	12	15	27	40	107	30	136	0	135	0
No. of experiment	—		—		11		12		13	

Notes.

In Nos. 12 and 13, after 171 minutes there was still some unclotted blood.

Many experiments of this nature have been carried out in this connection. Only one is given.

Experiments.

In these experiments, blood from the carotid through a paraffined cannula was received into paraffined vessels, as free from dust as possible. With 0·3 per cent. of "peptone" (dissolved in 0·9 per cent. sodium chloride) in the blood, again mixed *in vitro*, the delays in the commencement of clotting exceeded 23 minutes, as compared with the coagulation time on glass, a result which was not more marked than was obtained with 0·325 per cent. of "peptone" dissolved in 0·9 per cent. sodium chloride, mixed in an uncoated glass vessel. The distinctive difference between the results on glass and paraffined vessels is that, in the former, coagulation, once it has commenced, proceeds rapidly, and is completed in a few minutes, while in the latter it is a relatively slow process, occupying from 1 to 2 hours. In some cases, indeed, the first formed small clots are exhibiting syneresis (contraction), while other clots are still forming. Thus, in the less disturbed conditions of the paraffined vessels is the continuous inhibitory action of the "peptone" made evident.

A comparison of the coagulation times of pure blood on paraffined surfaces with those of blood mixed with "peptone" on similar surfaces shows less relative retardation in the time of commencement of clotting than is evident when the coagulation of pure and peptone blood in uncoated vessels is compared. The relative retardation in the time of completion of the clots is much more marked when blood mixed with "peptone" is in paraffined vessels than when it is contained in glass vessels not so treated.

In this connection, the partial failure of one experiment is noteworthy. The blood contained 0·8 per cent. of "peptone." It did not commence to clot until 31 minutes after the mixing of the blood and "peptone," but coagulation was complete 2 minutes later, a firm clot being formed which could be inverted without spilling.

This is a marked contrast to Experiments 11, 12 and 13, and cannot be ascribed to the higher concentration of "peptone" used, as typical slowing of clotting has been obtained after the appearance of the first clot in a number of cases where this and higher concentrations of "peptone" have been employed. In the specific case referred to, examination of the vessel containing the peptone blood revealed a small defect in the paraffin lining, with adherent fibrils to the exposed glass. Clotting had started from this point, and had spread through the liquid.

Attention is drawn to Experiment 13, where the "peptone" was 0·6 per cent. of the mixture; here clotting did not commence till 45 minutes 20 seconds, and was completed only as a semi-gel 2¼ hours after mixing. A small amount of the fluid was withdrawn by a pipette before coagulation was complete, and carbon dioxide passed through it. Clotting was observed after 10 minutes. This result was also obtained by dilution with distilled water. Other observations gave similar results. During the progress of the delayed clotting, portions of what remained fluid were, from time to time, withdrawn, and mixed with one-fourth of their volume of saturated ammonium sulphate. A precipitate of what was probably fibrinogen was always obtained (McLean, 39), till, at the end of the coagulation process, when the clots appeared to be complete, tests of the residual fluid yielded no precipitate. The mixture of "peptone" and blood *in vitro* thus behaved precisely as the blood peptonised *in vivo*.

Schmidt-Mulheim (*loc. cit.*) gives the dose of "peptone" necessary to produce incoagulability of circulating blood of dogs as from 0·3 to 0·6 grm. per kilogramme. Pollitzer (*loc. cit.*) found cats to be more resistant. Taking an animal weighing 3 kgrm., it may be assumed to contain 200 c.c. of blood. If 0·3 grm. per kilogramme of "peptone" is injected, the percentage in the blood is 0·45, or 0·9 if the larger quantity is used.

If, from a pithed cat which is breathing only air for at least 15 minutes, arterial blood is obtained through a paraffined cannula with only smooth surfaces exposed to the blood, then less than Schmidt-Mulheim's minimal dose for the dog will produce, in the more resistant cat, an unequivocal anticoagulant action *in vitro*, even in uncoated glass vessels. When the concentration of "peptone" is increased, but is still 33 per cent. less than Schmidt-Mulheim's larger amount, the results *in vitro* are parallel to those following the injection of "peptone" into the circulation of the living animal.

These data warrant the following conclusions:—

1. "Peptone," even in small quantities, acts directly on the constituents of the blood.

2. The apparent difference found by other observers between the action of "peptone" *in vivo* and its action *in vitro* is due to the changes towards clotting which take place in blood shed without special precautions to preserve its surface conditions.

3. It is superfluous to assume that the anticoagulant action of "peptone" *in vivo* is due to the secretion of antithrombin either by the liver or by the endothelial cells of the vascular system.

On the Supposed rôle of Leucocytes in the Anticoagulant Action of "Peptone."

Delezenne (40) considered that the first action of "peptone" on blood is leucolysis yielding two substances: *leuconuclein* which hastens coagulation and *leucohistone* which retards it. These both circulate through the liver where the former is retained. The latter remains circulating. Several workers including Halliburton and Brodie (41), Falloise (42) and Dastre and Studel (43) however deny that "peptone" on injection causes leucolysis. Even if this process does occur there appear to be no grounds other than pure speculation for the supposed liberation of these substances into the blood stream.

Nolf (44) maintained that under normal conditions an *unknown* substance is produced by the leucocytes which acts upon the endothelium of the liver and stimulates it to secrete antithrombin. The injection of "peptone" enhances this process and the excess of antithrombin accounts for the retarded coagulability of peptone blood.

Having demonstrated that the liver is not necessary for the production of the anticoagulant action of "peptone," it becomes of interest to enquire whether the leucocytes are concerned in this phenomenon. To this end Experiments 14–20 were devised.

Cats were bled through paraffined cannulæ into paraffined centrifuge tubes in ice and without delay these were centrifuged at 4° C., after which the tubes were again surrounded by ice till required. By this method a clear fluid mammalian plasma was obtained unaltered by foreign substances and which had made but little, if any, progress towards coagulation. At the temperature mentioned leucocytes do not exhibit amœboid movement and it may be assumed that all secretion by them is inhibited. To this plasma "peptone" was added in concentrations varying from 0·3–1·2 per cent. of the total volume of "peptone" and plasma and the mixture was observed at room temperature in paraffined vessels. In all cases except one, the typical anticoagulant action of "peptone" was evident. A study of the protocols shows that the results are essentially the same as when "peptone" in similar concentration is either added to shed blood at laboratory temperatures or is intravascularly injected into whole animals or into animals where the liver is out of circulation. Evidence is thus forthcoming that *leucocytes play no part in the inhibition of coagulation which follows the rapid admixture of blood and "peptone."*

	Control plasma on paraffin.		Control plasma on glass.		0·3 p.c. peptone plasma on paraffin.		0·6 p.c. peptone plasma on paraffin.		0·9 p.c. peptone plasma on paraffin.		0·9 p.c. peptone plasma on paraffin.	
	′	″	′	″	′	″	′	″	′	″	′	″
Time of adding "peptone" ...	0	0	0	0	0	0	0	0	0	0	0	0
Time of "commencement" of clotting	8	0	5	0	7	40	12	5	19	30	16	0
Time of "completion" of clotting	10	10	8	20	9	10	54	50	No end point		27	0
No. of experiment	—		—		14		15		16		17	

Notes.

Plasma obtained from animal respiring A.C.E. and air.

1. In No. 16 clotting was not typical; small clots were formed.
2. In No. 17 the gelatinous clot appeared to liquefy after 29 minutes; 10 minutes later all was liquid except one small clot. Syneresis is not excluded.
3. The residual fluids from 16 and 17 gave, next morning, no precipitate when fifth saturated with ammonium sulphate.
4. No. 14 is the only anomaly in a large number of observations.

A comparison of Experiments 14–17 with Nos. 18–20 illustrates the difference between the action of "peptone" on plasma from an animal under complete anæsthesia (A. C. E.) and the action of similar quantities of "peptone" on blood from a pithed animal 15 minutes after the discontinuance of the anæsthetic.

There is consistently less "peptone" inhibition of clotting when the animal is under anæsthesia at the time the blood is obtained. Early in these experiments it was evident that the shed blood of anæsthetised animals was

	Control plasma on paraffin.	0·6 p.c. peptone plasma on paraffin.	0·9 p.c. peptone plasma on paraffin.	1·2 p.c. peptone plasma on paraffin.
	′ ″	′ ″	′ ″	′ ″
Time of adding peptone	0 0	0 0	0 0	0 0
Time of " commencement " of clotting	15 0	34 45	50 20	24 30
Time of " completion " of clotting	23 50	41 45	51 20	30 0
No. of experiment	—	18	19	20

Notes.

1. Repetition of Nos. 18 and 19 gave the same results.
2. In No. 20, after 20 minutes the gel appeared to liquefy, due probably to syneresis.
3. Plasma was obtained from animal respiring air.

more prone to coagulate rapidly than that of pithed animals 15 minutes after respiring only air. For this reason, except for the experiments cited above, all observations not on pithed animals were discarded.

In both the last two series of experiments typical syneresis was exhibited in the clots formed. The view that blood platelets are necessary for the production of syneresis (Bordet and Delange, 45) may be mentioned.

The Influence of Speed of Injection on the Action and Immunity to the Action of Peptone on Blood.

Fano (46) found that, if "peptone" is injected slowly into the circulation, it has not any anticoagulant action on the blood, and later Nolf (*loc. cit.*) showed the concentration of "peptone" as well as the speed of its injection to be the determining factors either in the production of anticoagulant action or complete or partial immunity thereto. Nolf explained the immunity following minimal doses injected rapidly and larger doses injected slowly by suggesting that leucocytes are not acted upon by "peptone" if delivered sufficiently slowly, and that they become accustomed to increasing doses. The leucocytes, he believes, under these conditions fail to produce the *unknown* substance which stimulates the liver to secrete either antithrombin or fibrinolysin. The experiments described in the preceding section demonstrate that leucocytes are not essential for the production of the anticoagulant action of "peptone" *in vitro*, and there appears to be no valid reason for assuming their presence to be necessary for the production of the same effect *in vivo*, when the concentrations are strictly comparable.

In sharp distinction to the generally accepted view is that of Mellanby (47), who maintained that fluidity of peptone blood is due to the secretion of an excess of alkali by the liver, under the influence of a stimulus of injected "peptone," and the "peptone" immunity is caused by the temporary disappearance from the liver of the excess of alkali secreted under the toxic stimulus.

The next experiment, No. 21, shows that if relatively large amounts of "peptone" are injected slowly, in minimal doses, over a long period of time, into a cat in which the circulation through the liver has been prevented, then, as in the intact animal, no anticoagulant action is to be obtained.

Experiment No. 21.—Cat, 3¼ kilos. Pithed, artificial respiration. Aorta ligatured beyond the coronary arteries, inferior vena cava above the diaphragm.

Over period of 1 hour 10 minutes 1·8 grm. of "peptone" in 0·9 per cent. NaCl was injected in nine equal amounts, each injection being 2·5 c.c. in volume and being made at approximately equal intervals of time.

The coagulation times of the blood was taken at frequent intervals during the injection of the "peptone" and at no time showed any delay.

Control Blood on Glass.—Commencement of clotting, 9 minutes; completion of clotting, 12 minutes 10 seconds.

The total amount administered was 1·8 grm., and as only about one-fourth of the animal's blood was circulating, the concentration of "peptone" in the blood at the end of the experiment may be estimated at 3·6 per cent., an amount much in excess of that required to produce anticoagulant action both in whole cats and in cats in which the liver is not acting, provided that such an amount is injected slowly. Moreover, reference to Experiment No. 10 shows that a concentration of 3·5 per cent. of peptone in the blood *in vitro* produced incoagulability over a period of 24 hours. Assuming in the *in vivo* experiment mentioned above that the quantity of blood dealt with was 50 c.c., a concentration of "peptone" exceeding that required to produce prolonged incoagulability *in vitro* was injected without anticoagulant effect.

Some other explanation must therefore be sought for this immunity to the slow injection of "peptone" than is supplied by the divergent views of Nolf and of Mellanby. These hypotheses have one point in common, they assign the cause of immunity to hepatic secretion, and it is precisely this point of both theories which is shown to be untenable by the experiments given above. Whether the alkali reserve or the hydrion concentration of the plasma is concerned in these occurrences must be left for future determination. There are, however, some indications which point in this direction. Fano (*loc. cit.*) showed that peptone blood could be clotted either by the passage through it of carbon dioxide or by neutralisation by acid. Earlier in the present paper the influence of the concentration of carbon dioxide in circulating blood on the action of "peptone" has been emphasised. It is noteworthy that Mills (48) has recorded that a diminution of the alkali reserve occurs *pari passu* with the coagulant action arising from the intravenous injection of lung extract. It is well known that the advent of asphyxia will determine the appearance of thrombosis after the injection of such amounts of kidney nucleoprotein as, in the presence of normal amounts of carbon dioxide, tend to produce incoagulability or immunity. In like manner Halliburton and Pickering (49) showed that an increase in the concentration of the carbon dioxide of the blood will annul the "negative phase" resulting from the injection of minimal doses of these synthesised substances which have been shown to be intravascular coagulants (Pickering, 50).

Still more significant is the correlation of all these "negative phases" and temporary immunities to the factors of the speed of action and concentration of the toxic substances. In this respect they are similar to the "negative phases" which occur *in vitro* alluded to earlier in this paper.

There is a physical or physico-chemical interpretation indicated.

An application of the theorem of Le Chatelier (51) may provide a more satisfactory explanation than is afforded by current hypotheses.

On Experiments with perfused Livers.

It is commonly held that perfusion of the liver affords evidence of the secretion of antithrombin by that organ. Delezenne (52) and later Nolf (53), who had doubtful results, found that perfusion of the liver with a mixture of defibrinated blood and "peptone" gave an incoagulable liquid which, when added to shed blood, retarded its coagulation. Apparently these workers were unaware of earlier observations of Pavloff (54), who had found that the circulation of a mixture of defibrinated and normal blood through a heart-lung preparation yielded a fluid which remained uncoagulated for several days. Work is now in progress which indicates that certain products of autolysis may act as anticoagulants, and in view of Pavloff's experiments it is superfluous to assume that Delezenne's results were due to hepatic activity. Doyon (55) connected the carotid of one dog with the portal vein of another, and examined the coagulation times of blood from the inferior vena cava. With young fasting dogs the first perfusates coagulated normally, but later samples showed delay in coagulation. With adult dogs, even after fasting, no retardation of clotting was observed. It is well known that the blood of embryos is resistant to clotting and these isolated experiments only afford evidence of a similar phenomenon in the blood of young fasting dogs.

Summary and Conclusion.

1. The retardation of the coagulation of the blood resulting from the rapid intravascular injection of "peptone" into either whole cats or into cats with the liver out of circulation is reduced or annulled by increase of carbon dioxide in the blood.

2. The anticoagulant action of "peptone," if annulled by an increase of carbon dioxide, can be restored by administration of oxygen or of excess of air.

3. In pithed animals respiring air, the retardation of coagulation of shed blood following the rapid injection of moderate amounts of "peptone," can be produced when the liver is out of the circulation.

4. Previous failures to obtain this result are due to attention not having been paid to the increase of carbon dioxide in the blood of animals with impaired vitality following operation and prolonged narcosis.

5. With suitable precautions to preserve the surface conditions of the blood, typical "peptone" delays of coagulation can be obtained *in vitro* with quantities of "peptone" no greater than are required to produce a similar effect in the living animal.

6. If special precautions are not taken to preserve the surface conditions of shed blood, admixture *in vitro* with moderate amounts of "peptone" causes no appreciable anticoagulant effect.

7. Leucocytes play no part in the anticoagulant action of "peptone" on blood.

8. It is superfluous to assume that the anticoagulant action of "peptone" on blood is due to hepatic secretion of an excess of alkali under the toxic stimulus.

9. It is unnecessary to assume that the anticoagulant action of "peptone" is due to the secretion of antithrombin, either by the liver or by other cells of the body.

10. Typical immunity to the action of "peptone" on blood can be obtained by injecting maximal amounts, in repeated small doses, into animals with the liver out of circulation.

11. The current hypotheses on "peptone" immunity are shortly discussed, and reasons given for not accepting them. Immunity to "peptone" appears to be a physical process akin to adsorption.

12. Experiments on the perfusion of the liver do not show that antithrombin is normally secreted by that organ.

13. If the foregoing conclusions are correct, it follows that in the interpretation of the coagulation of the blood it is unnecessary to assume the existence of *antithrombin*, *pro-antithrombin*, and *anti-prothrombin*.

14. If antithrombin is not a normal secretion of the liver, some explanation of blood coagulation, other than the current " thrombin theories," is necessary.

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Active Hyperæmia.

By D. T. Harris (Beit Memorial Research Fellow).

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1. Introduction.

So conflicting are the statements made by physiologists and pathologists alike, relating to the mechanism by which "an increased afflux of blood to a part" is brought about, that it was felt desirable to investigate this important phenomenon. Let us first consider the present state of knowledge regarding the possible factors involved.

(i) *Vaso-constrictor Nerves* (neuro-paralytic hyperæmia).—The existence of vaso-constrictor nerves was first demonstrated in the cervical sympathetic by Claude Bernard (1852), and independently by Brown-Séquard. The interruption of the normal stream of impulses from the vaso-motor centres along these nerves gives rise to a "neuro-paralytic hyperæmia," such as is met with surgically in wounds of the neck, or in lesions of the spinal cord. During the